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TECHNICAL MANUSCRIPT T12

DISINFECTION OF
AEROSOLIZED PATHOGENIC FUNGI
ON LABORATORY SURFACES.

II. CULTURE PHASE

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UNITED STATES ARMY
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TECHNICAL MANUSCRIPT 112

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FUNGI ON LABORATORY SURFACES.

II. CULTURE PHASE

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ABSTRACT

The effect of several fungicides on laboratory surfaces contaminated with the culture (spore) phase of aerosolized Blastomyces dermatitidis, Coccidioides immitis, and Histoplasma capsulatum was ascertained. The culture (spore) phase was more resistant to the action of the fungicides than the tissue (yeast) phase. The addition of a wetting agent increased the efficiency of several fungicides. The time required for disinfection with a given concentration of fungicide, or the concentration required to disinfect within a given time, can be determined by interpolating the plotted graphs.

I. INTRODUCTION

Part I of this study¹ dealt with the effect of several fungicides on laboratory surfaces contaminated with the tissue (yeast) phase of aerosolized pathogenic fungi. Conditions that may occur when infectious aerosols are created accidentally by mycological laboratory techniques were approximated. This work has been continued with the cultural (spore) phase of Blastomyces dermatitidis, Coccidioides immitis, and Histoplasma capsulatum, using the test procedures described in Part I.

II. MATERIALS AND METHODS

A. TEST FUNGI

B. dermatitidis 3110, C. immitis Silveria, and H. capsulatum 3021 were grown on Mycophil agar* at 30°C for approximately 30 days. When microscopy showed optimum spore production, the culture plates were placed in a drying chamber containing Drierite** at 39°C to promote desiccation of the agar. When desiccation was complete, the dried spores were collected by a vacuum-extraction thimble apparatus.² Purity, viability, and concentrations of the harvested spores were ascertained by microscopy and serial dilutions.

B. TEST SURFACES

Experiments were begun using the same five experimental surfaces and the methods described in Part I. However, there was no growth on either the controls or the test surfaces, which indicated that the spores did not attach to the surfaces firmly enough for purposes of the test. Consequently, a revised test method was adopted.

C. REVISED METHOD

The procedure is diagrammed in Figure 1. Sterile one-square-inch sections of the five test surfaces were placed in an aerosol chamber. One milligram of dried spores was placed in a No. 5 gelatin capsule. The test fungi were aerosolized in the chamber by placing the capsule in a CO₂ pistol that was modified to burst the capsule and create an homogeneous aerosol.

* Baltimore Biological Laboratories, Baltimore, Maryland.

** W. B. Hammond Drierite Co., Xenia, Ohio.

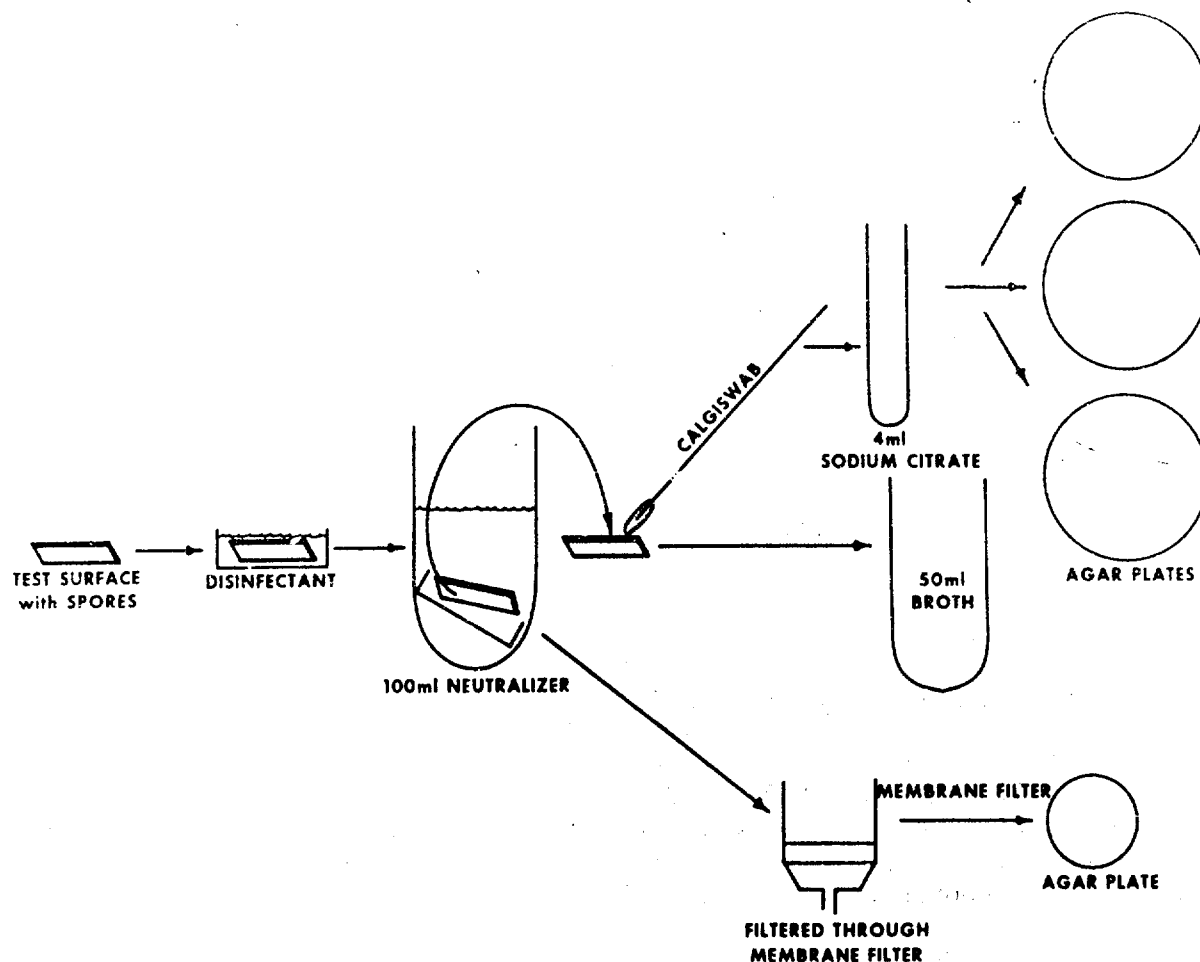


Figure 1. Diagram of Test Procedure.

Each seeded surface was then immersed in a stainless steel planchet containing the test disinfectant. At 1, 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes, the planchet containing the surface section and disinfectant was shaken gently for 30 seconds in 100 milliliters of an aqueous solution of a neutralizer that was specific for the test disinfectant.

The section of surface was then removed from the neutralizer, swabbed with a Caligswab,* and immersed and shaken in 50 milliliters of broth (PD broth) containing one per cent Phytone** and one per cent dextrose. The 100 milliliters of used neutralizer solution was passed through a membrane filter.

* Consolidated Laboratories Inc., Chicago Heights, Illinois.

** Baltimore Biological Laboratories, Baltimore, Maryland.

The Caligswab was dissolved in four milliliters of one per cent $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, and 0.5-milliliter samples of the resulting suspension were plated in triplicate on Mycophil agar containing 100 units of penicillin, 125 micrograms of streptomycin, and 0.5 milligrams of actidione per milliliter.

The PD broth tubes, the used membrane filters (on a Mycophil agar plate), and the swab-suspension Petri plates were incubated at 30°C for 14 days.

A control was tested concurrently by immersing the seeded surface in sterile 0.85 per cent NaCl and proceeding as with a test surface. Growth occurred in all controls.

D. CANDIDATE FUNGICIDES AND NEUTRALIZING SOLUTIONS

The same fungicides and neutralizing solutions employed in Part I were used. Because the dried spores, and dried microorganisms in general, tend to be lyophobic, comparative studies were done with and without the addition to the test fungicide of 0.01 per cent wetting agent.* The specific neutralizer was added to the distilled water used to prepare the PD broth.

III. RESULTS

A. STATISTICAL EVALUATION

As with the tissue (yeast) phase, plots for each fungus fungicide combination were linear when "log time required for disinfection" was plotted against "log concentration of disinfectant." For a given concentration, the best estimate of time required for disinfection is obtained from the midpoint of the logarithm of the shortest time at which disinfection occurred and the logarithm of the next highest time at which there were positive results. Linear regressions of these midpoints on log concentration were computed, and the parameters of regression are shown in Table I.

It was next desired to know whether these regressions could be treated as parallel. It was anticipated that parallelism might not exist between fungi because of the different physical and chemical characteristics of the organisms. The hypothesis of parallelism among fungicides for each fungus was tested. It should be noted that variances were computed from interpolated points; however, the variation involved in the interpolations

* Igepal CO-630; Antara Chemicals, New York 14, N. Y.

TABLE I. REGRESSION PARAMETERS FOR LOG Y = a + b (LOG X)

Fungicide	Coccidioides immitis		Histoplasma capsulatum		Blastomyces dermatitidis	
	Parameter b, Slope	Parameter a, Intercept	Parameter b, Slope	Parameter a, Intercept	Parameter b, Slope	Parameter a, Intercept
Phenolic A	0.7533	1.5047	0.6787	1.2307	0.8501	0.9032
Phenolic B	0.4725	1.7132	0.4698	1.4032	0.9237	1.3216
Cresylic	0.8970	1.0970	0.8910	0.7987	0.9058	0.8030
Quat	0.7715	1.5948	0.9172	1.4072	0.8501	0.9032
Iodenic	1.0471	1.2133	0.8910	0.7987	0.8028	0.8413
Phenol	0.7752	1.6701	1.0243	1.4967	1.2299	1.4085
Phenol with Igepal	0.8268	1.5830	1.2299	1.4085	1.1876	1.3514
Hypochlorite	0.4660	1.7081	0.7751	1.5948	0.9278	1.5062
Hypochlorite with Igepal	0.9052	1.6974	1.0761	1.5602	0.9237	1.3216
Formaldehyde	1.0761	1.6354	0.7820	1.3010	0.9172	1.4072
Formaldehyde with Igepal	1.0761	1.5602	0.8716	1.2436	1.1820	1.0722
Peracetic acid	0.9058	0.8030	1.0604	0.5348	0.8087	0.6909

themselves could not be estimated. With this limitation, the analyses of variance shown in Table II were computed. The significance level for the test of variation among slopes was extremely low for C. immitis and H. capsulatum, indicating that for these two fungi the slopes could not be considered parallel. For B. dermatitidis the significance level approached 0.05, but when the common slope was applied, the computed regression lay outside the observed time intervals for many of the individual fungicides. As the observed time intervals were accurately determined, individual regression parameters were used for each fungus fungicide combination.

Analysis of experiments led to the following conclusions:

(a) The model $Y = 10^a X^b$ or $\text{Log } Y = a + b (\text{Log } X)$, where Y is the time required for disinfection, X is the concentration of disinfectant, a is the intercept, and b is the slope, accurately represented the regression of "time required for disinfection" on "concentration of disinfectant" for all disinfectants except ethyl alcohol.

(b) Contrary to the tissue (yeast) phase, the parameter b of the culture (spore) phase could not be treated as constant, and individual regression estimates were required for each fungus fungicide combination.

(c) The regression of time for disinfection on concentration of disinfectant on ethyl alcohol was fitted to additional observed results rather than by a computed equation, because ethyl alcohol did not fit the model.

B. CORRELATION WITH PERTINENT REPORTS BY OTHER INVESTIGATORS

Bartlett and Schmidt,⁶ comparing fungicidal activities on Trichophyton interdigitale and Penicillium luteum, have shown iodenic compound to be more fungicidal than hypochlorite and quaternary ammonium compound. Lawrence, Carpenter, and Naylor-Foote⁴ compared the fungicidal activity of an iodenic compound with the activity of a quaternary, a cresylic, and a phenolic substance, on several dermatophytes. They concluded that the iodenic compound was more fungicidal than the quaternary ammonium compound. The iodenic and cresylic compounds were similar in their fungicidal efficiency. Klarmann, Shternov, and Gates⁵ found that halogen substitution of phenol intensified its microbial potency, inasmuch as T. rosaceum was killed in 10 minutes by concentrations of chlorinated phenols ranging from 0.001 to 0.1 per cent, whereas it was necessary to use one per cent phenol to effect the same result.

TABLE II. ANALYSES OF VARIANCE OF SLOPES

Fungus	Source	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratios	Significance Level
<u>Coccidioides immitis</u>	Regression due to \bar{b}	1	8.11235			
	Variation among b's	12	0.33003	0.02750	10.7	0.001
	<u>Pooled residual</u>	<u>29</u>	<u>0.07431</u>	<u>0.00256</u>		
	Within fungicide	42	8.51659			
<u>Histoplasma capsulatum</u>	Regression due to \bar{b}	1	7.97204			
	Variation among b's	12	0.37958	0.03163	9.6	0.001
	<u>Pooled residual</u>	<u>31</u>	<u>0.10216</u>	<u>0.00330</u>		
	Within fungicide	44	8.45378			
<u>Blastomyces dermatitidis</u>	Regression due to \bar{b}	1	13.12001			
	Variation among b's	12	0.23866	0.01989	1.97	0.05
	<u>Pooled residual</u>	<u>37</u>	<u>0.37450</u>	<u>0.01012</u>		
	Within fungicide	50	13.73317			

It is our opinion that the techniques found in literature for testing fungicides are basically the same as those used for testing bactericides. In the referenced studies, tests were carried out with dermatophytes, usually suspended in liquid menstruum. Stuart⁶ stated that little or no reliable information has been developed regarding other pathogenic fungi. Simulation of laboratory techniques and accidents, using aerosolized pathogenic fungi, may cause infection of the experimenter. However, by doing the experiments in a ventilated, gas-tight cabinet system, tests can safely be performed that simulate deposition of aerosolized fungal particles on various laboratory surfaces.

IV. DISCUSSION

All fungicides tested, given the proper contact time and concentration, were effective against B. dermatitidis, C. immitis, and H. capsulatum spores. In the previous study⁷ it was shown that the nature of the test surface is an important factor in determining the efficacy of fungicides. When dried spores were aerosolized, the nature of the surface was not a determining factor because each fungus acted essentially the same on all the test surfaces. The heterogeneity in fungicidal resistance among the fungi is evident in Tables III and IV. The action of a fungicide on varying spore walls produced a logarithmic gradation of resistance. Emmons⁸ stated that different pathogenic fungi vary in their response to various disinfectants. Whether this is caused by differences in permeability of the cell wall and subsequent action on the cytoplasmic or nuclear matrix is not known.

The nature of respective interfaces dominates the phenomena of disinfection. The wetting power may depend on surface tension, nature of the fungicide, or chemical constituents of the spore. Stedman, Kravitz, and Bell⁹ reported that surface tension could affect bactericidal activity in immersionsal wetting by affecting the concentration at the solid-liquid interface governed by Gibb's equation. The addition of a wetting agent to formaldehyde, phenol, and hypochlorite lowered the solid-liquid free energy. The wetting agent was adsorbed at the interface¹⁰ and decreased the time and concentration needed to disinfect (Table III).

Various reports state that quaternaries are less fungicidal on spores than on yeast. In Part I, Quat was statistically ranked higher than most of the fungicides in its effect on the tissue (yeast) phase. But the current study, Part II, showed Quat to be less effective against the culture (spore) phase. In both Part I and Part II, peracetic acid was the most effective fungicide.

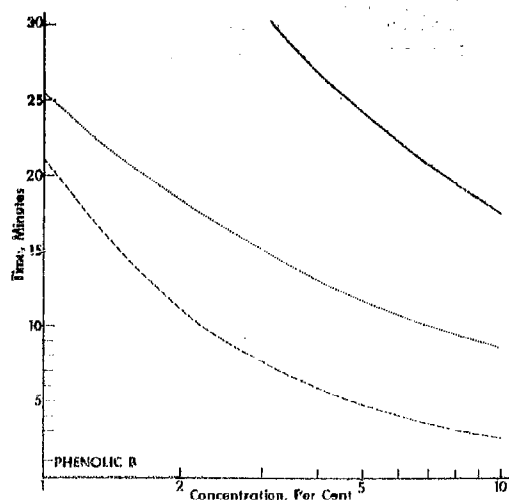
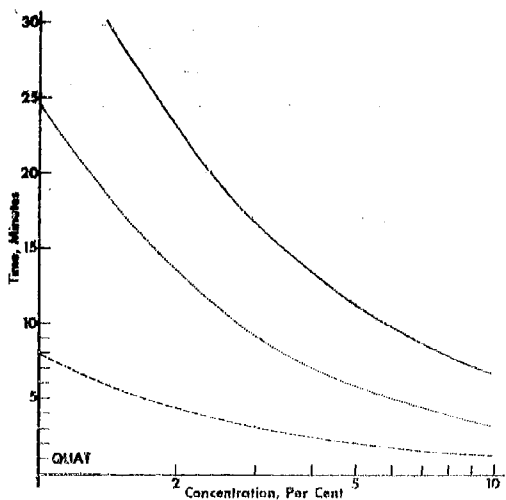
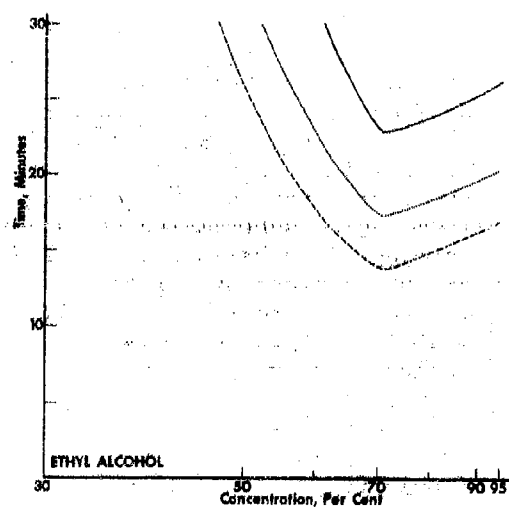
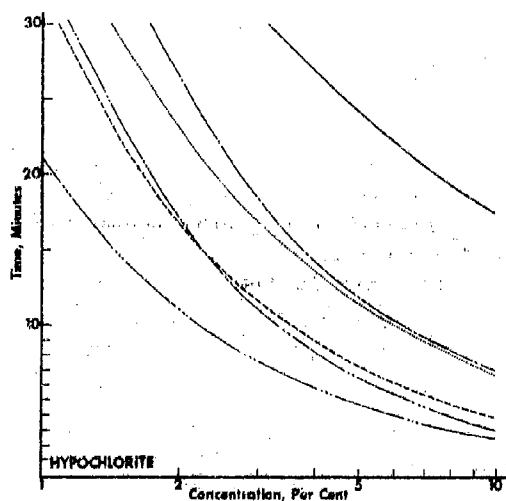
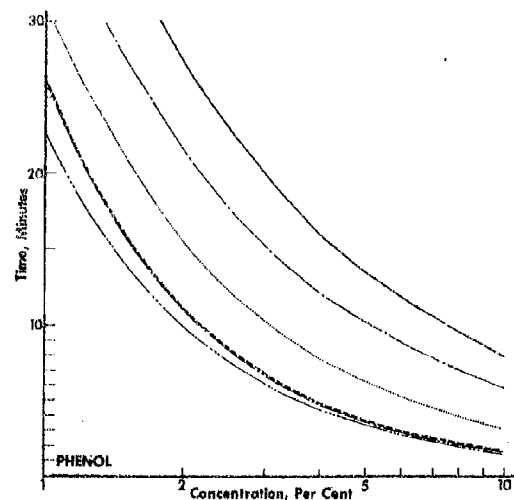
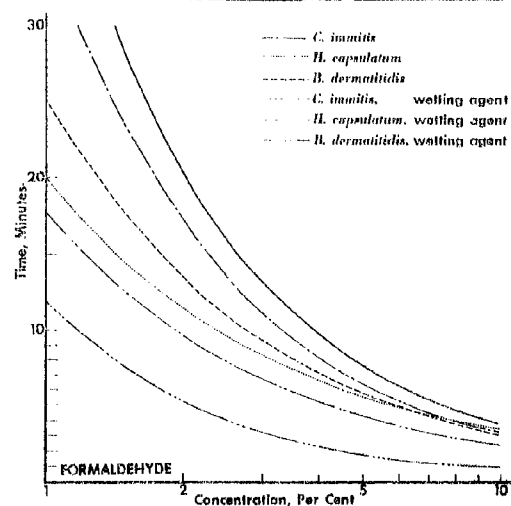
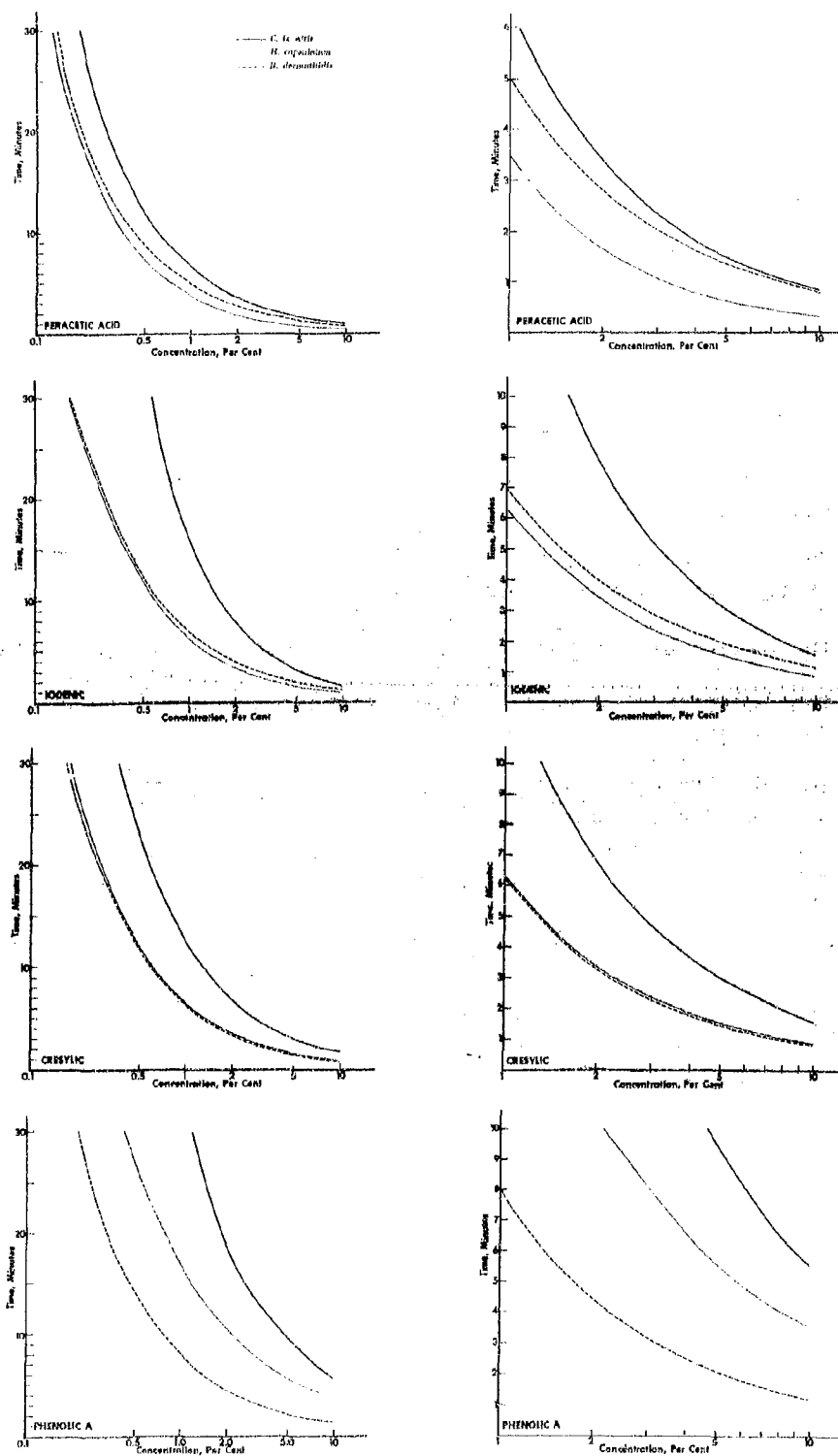
TABLE III. EFFECT OF FUNGICIDES ON *COCCIDIODES IMMITIS*,
HISTOPLASMA CAPSULATUM, AND *BLASTOMYCES DERMATITIDIS*

TABLE IV. EFFECT OF FUNGICIDES ON COCCIDIOIDES IMMITIS,
HISTOPLASMA CAPSULATUM, AND BLASTOMYCES DERMATITIDIS



The fungicides shown in Table III required higher concentrations and more time to disinfect the surfaces than did those shown in Table IV. To make it easier to determine the disinfecting efficiency by graphic interpolation, Table III was prepared on one log cycle (1.0 to 10 per cent) and Table IV was prepared on two log cycles (0.1 to 10 per cent). For those fungicides requiring two log cycles, an additional graph of one log cycle was devised as shown in the right side of Table IV, with distance for time enlarged, to assist in interpolating more accurately (a) time required for disinfection with a given concentration of fungicide, or (b) concentration required to disinfect within a given time.

V. SUMMARY

1. All fungicides tested killed the culture (spore) phase of B. dermatitidis, C. immitis, and H. capsulatum.
2. The culture (spore) phase was more resistant to the action of the fungicides than the tissue (yeast) phase.
3. The nature of the surface did not influence fungicidal activity.
4. The addition of a wetting agent increased the efficiency of several fungicides.
5. Interpolation in Tables III and IV will reveal the times and the concentrations of each fungicide required for disinfection.

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